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Housing conditions influence cortical and behavioural reactions of sheep in response to videos showing social interactions of different valence

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Abstract

Mood, as a long-term affective state, is thought to modulate short-term emotional reactions in animals, but the details of this interplay have hardly been investigated experimentally. Apart from a basic interest in this affective system, mood is likely to have an important impact on animal welfare, as bad mood may taint all emotional experience. In the present study about mood - emotion interaction, 29 sheep were kept under predictable, stimulus-rich or unpredictable, stimulus-poor housing conditions, to induce different mood states. In an experiment, the animals were confronted with video sequences of social interactions of conspecifics showing agonistic interactions, ruminating or tolerantly co-feeding as stimuli of different valences. Emotional reactions were assessed by measuring frontal brain activity using functional near-infrared spectroscopy and by recording behavioral reactions. Attentiveness of the sheep decreased from videos showing agonistic interactions to ruminating sheep to those displaying co-feeding sheep. Seeing agonistic interactions was also associated with a deactivation of the frontal cortex, specifically in animals living under predictable, stimulus-rich housing conditions. These sheep generally showed less attentiveness and locomotor activity and they had their ears in a forward position less often and in a backward position more often than the sheep from the unpredictable, stimulus-poor conditions. Housing conditions influenced how the sheep behaved, which can either be thought to be mediated by mood or by the animals' previous experience with stimulus-richness in their housing conditions. Frontal cortical activity may not depend on valence only, but also on the perceptual channel through which the stimuli were perceived.

Keywords: emotion, mood, attentiveness, social interaction, functional near-infrared spectroscopy (fNIRS), sheep

1 Introduction

Affective states likely play an important role in the proximate control of behaviour not only in humans (e.g. [1]) but also in animals. Long-term mood and short-term emotions are often differentiated [2,3], but the interplay of these types of affective states has hardly been addressed. While Mendl et al. [2] assumed mood to be influenced by repeated, recent emotional experiences, Reefmann et al. [4] found that emotional reactions to specific stimuli may in turn be modified by the mood state of an animal. This interplay is not only of theoretical interest but also of importance in respect to animal welfare. For example, it seems relevant for the assessment of welfare whether negative mood will taint all experiences, as it was found in studies of human depression [5,6]. In contrast, positive mood was found to mitigate short-term negative and positive experiences as seen in weaker behavioural and physiological responses to emotional stimuli [4].

Emotions are triggered often by external stimuli [7,8]. These stimuli vary in valence, that is, they are perceived as differing in their position on an axis ranging from negative to positive [2]. While humans can express their subjectively perceived emotions verbally, indicators are needed to assess emotional states in animals [9,10]. Locomotor activity [11], restlessness behaviour [12], and ear postures and movements [11-15] have been used as behavioural indicators of animal emotions in recent studies. These studies related increased locomotor activity and restlessness behaviour with negative stimuli. Furthermore, increased ear movements and specific ear postures were found with negative situations [13,14,16] and possibly mediated by the animals' attention [11,14,17].

As emotional reactions are processed in the brain, measurements of changes in frontal brain activity are another promising indicator to assess emotions in animals [18]. Functional near-infrared spectroscopy (fNIRS) has emerged as a reliable measurement technique of neuronal cortical activity in animals [18,19]. It measures non-invasively the haemodynamic changes in the brain with a wireless application that allows measuring freely moving animals.

To evoke a broad range of emotional reactions in experimental studies, the animals need to be confronted with different types of stimuli varying in their valence. To vary valence and at the same time as few other aspects of the stimuli, such as arousal, as possible, it seems useful to remain within one sensory channel in a given experiment (e.g. [12]). Emotional reactions investigated in previous studies include physical stimuli [12,18] and situations with ambiguous information [20] in sheep, feed and feed frustration in goats [19] as well as interactions with a human handler in dogs [21]. In all these studies an attempt has been made to vary valence of the stimuli to a certain extent. If reactions towards stimuli of a specific valence are consistent independent of the specific situation and the specific sensory channel, this is strong support that the observed reactions are specific for a given valence and allow for more general statements in respect to the typical reaction in response to emotional stimuli. In the present study we extend the classes of stimuli by using social interactions that are visually perceived. The various types of social interactions presumed to have different emotional valence for the animals were presented with the help of video sequences. This method is used often to evoke a specific reaction in animals [22] and allows presenting different scenes within a short period of time. We expected that video sequences showing agonistic interactions like fights (i.e. head butts, displacements) have a negative valence for the observing sheep. In contrast, video sequences of affiliative interactions like co-feeding of two animals were presumed to be of positive valence. Video sequences showing a ruminating sheep were assumed to be of intermediate valence, with little relevance for the observing conspecific.

An accumulation of emotional experiences with similar valence can lead to a distinct mood state [2], either negative or positive. It has been shown that manipulations of the animal's housing system can lead to such an accumulation and can therefore change mood. For example, the unpredictability of events, such as the provision of feed (e.g. [23,24]), induces a more negative mood state, while an increased amount of stimuli in the housing environment (e.g. [25,26]), a higher space allowance (e.g. [26,27]) and the predictability of events (e.g. [23]) induce a more positive mood state as assessed in cognitive judgement bias tests.

In the present study, we kept sheep under either unpredictable, stimulus-poor or predictable, stimulus-rich housing conditions to induce differential mood states. Emotional reactions were elicited by presenting videos of fighting, ruminating and co-feeding animals. We used brain activity and behavioural parameters as indicators for emotional reactions to examine potential differences between the stimuli differing in presumed valence and between the housing conditions. We expected more general activity, more ear movements and a stronger frontal brain activity in response to the presumed negative stimulus (i.e. the presentation of agonistic interactions) than to the presumed positive and neutral stimulus. Furthermore, we expected that emotional reactions would be stronger in animals living under unpredictable, stimulus-poor housing conditions compared with the animals living under predictable, stimulus-rich housing conditions.

2 Materials & Methods

2.1 Animals

Twenty-nine non-lactating and non-reproducing Lacaune ewes, born between November 2010 and March 2011, were housed in two separate housing systems (14 and 15 randomly allocated sheep; described in 2.3) at the Agroscope Research Station in Tänikon, Switzerland. For the experiments, which took place between September and October 2012, 12 focal sheep of about 20 months of age from each of two housing groups were chosen to allow for full sets of all possible sequences of the presentation of three stimuli (6 possible sequences; see 2.4) and to reach a sample size (2 x 12) which provided sufficient power to detect such differences in previous studies [12,18]. These were the same focal sheep that had been tested in a previous experiment using physical stimuli [12].

2.2 Ethical note

All experiments were performed as part of a project funded by the Swiss National Science Foundation and had the necessary cantonal authorisation to conduct animal experiments (Canton of Thurgau permit nos. F6/10 and F4/11).

2.3 Mood induction

We aimed at inducing different mood states by housing animals under two different housing conditions, namely a predictable, stimulus-rich and an unpredictable, stimulus-poor pen described in detail by Vögeli et al. [12]. In short, the predictable, stimulus-rich housing conditions included an open-front pen (58.0 m^2) which had a separated feeding (concrete floor) and lying area (deep litter) with an outdoor exercise yard (concrete floor) and regular access to pasture. Feed was provided twice a day at regular times (between 07.30 and 08.00 a.m. and between 04.30 and 05.00 p.m.). Sheep had permanent access to water and were exposed to the normal daylight cycle. Under unpredictable, stimulus-poor housing conditions, sheep were living in an unstructured, enclosed deep-litter pen (22.4 m^2; conforming to the Swiss minimal legal standards) with no possibility to leave the pen and no visual cues from outside the pen. Access to food, water and daylight was granted daily at irregular times [12].

Nine weeks before the start of the experiment presented here and after being kept about 10 months in their respective housing conditions, a cognitive judgement bias test was performed to compare mood between the animals of the two housing groups [12]. Results showed that sheep from the unpredictable, stimulus-poor housing conditions had a slightly more negative cognitive bias and needed a longer period of learning compared with the sheep from the predictable, stimulus-rich housing conditions.

After the cognitive judgement bias test was completed, the animals lived again under constant and undisturbed conditions as described above for six weeks before the experiment of the current study.

2.4 Emotional stimuli

To evoke an emotional reaction in the sheep, three stimuli differing in their presumed valence were used. The stimuli were presented as silent video images recorded several weeks before testing. They all showed sheep from the focal sheep's group engaged in social interactions. Videos showing a dyad of sheep engaged in head butts or displacements with body contact were used for the presumed negative valence (following the argument of research on agonistic interactions in species as diverse as geese [28], chimpanzees [29], and humans [30,31,32]). For an intermediate valence, videos with one or two sheep ruminating while lying were presented. Videos showing two sheep tolerantly co-feeding from the same bowl were used for the presumed positive valence (following the interpretation of the effect of affiliative interactions in chimpanzees [29] and humans [30,31,32]) . Each stimulus video sequence lasted 20 s and consisted of three to four short tracks of single social interactions (4.0 to 8.5 s) to keep the sheep's continuous attention towards the pictures. Stimulus videos of each type of valence were repeated seven times per animal in direct succession, resulting in 21 stimulus videos in total for all three valences. In each repetition, different single tracks were used or tracks were combined in a different order depending on the available number of single tracks to avoid habituation and to cover a broad range of stimuli with the same valence. The video sequences were the same for all sheep from one housing group but differed between the housing groups. Each sheep was confronted with all three valences on one day, and the order of the three valences was balanced across sheep such that each of the six possible sequences for the three valences was used for two sheep from each housing group.

The inter-stimulus interval between the presentations of the different stimulus video sequences varied randomly in duration (35 to 45 s) to avoid anticipation. A pre-stimulus interval lasting 20 s preceded the first stimulus video sequence, and a post-stimulus interval lasting 20 s followed the last stimulus video sequence. In these intervals, a black screen was presented. Videos were projected with a projector (ASK Dataview S240) onto one side wall (painted white; Fig. 1) of a test pen (see section 2.8) and presented such that the sheep in the video were about life-sized. The complete video films including the pre-, inter- and post-stimulus intervals were embedded in a Microsoft PowerPoint (version 2010) presentation.

2.5 Frontal brain activity

All three types of stimulus valence were assumed to elicit neuronal activity in the brain. This activation was measured by functional near-infrared spectroscopy (fNIRS), which enables measuring haemodynamic changes in the brain, namely the changes in oxy- and deoxyhaemoglobin concentrations ([O_2Hb] and [HHb]; [18]). The wireless fNIRS device (7.0 cm x 4.5 cm) consisted of two detectors and four light sources emitting infrared light at two wavelengths (LED at 760 and 870 nm) each (16 light paths in total; source-detector distances of 14 and 22 mm; [33]). In the evening before the test, each sheep's head was shaved and depilated to avoid any interference between the sensor and hair (light-piping). Immediately before the test, the sensor was placed towards the frontal part of the animal's head to measure as effectively as possible the activity in the frontal cerebral cortex.

Due to the alignment of the light sources and detectors, a potential localization of the concentration changes (right/left, caudal/cranial and shallow/deep) was detectable by the sensor. The original sampling rate was 100 Hz, but the data were filtered resulting in relative [O_2Hb] and [HHb] values at 1 Hz.

2.6 Behavioural measurements: restlessness behaviour and attentiveness

During the presentation of video sequences, the occurrence of several types of behaviour was counted directly by one of two experimenters using hand tally counters (HC-2, Voltcraft, Hirschau, Germany). Each experimenter counted specific behaviour patterns throughout all tests. The following behaviours were differentiated: aversive behaviour (including shaking, stamping, bucking, and moving backwards), vocalisations, rearing up the walls and nibbling (for the detailed definitions, see [12]). These behaviour patterns were shown very rarely such that no quantitative evaluation was possible.

Since animals could move freely in the test pen, they were not always directly facing the video screen. However, their specific attentiveness towards the video sequences was quantified. Based on direct observations, we noted for each of the seven stimulus sequences per valence whether the sheep had their head turned to the screen, that is, the axis of their head deviated at most 90 degrees from the head to screen axis (head parallel to the screen), for at least 2 seconds ('being attentive'). This is a conservative estimate of the sheep's attention because their visual angle is up to 313 degrees [34] and our measure may therefore reflect rather an active interest than pure exposure only.

2.7 Behavioural measurements: locomotor activity and ear postures

Ear movements and postures were recorded during the test by an automatic tracking system (Trackpack4, Advanced Realtime Tracking GmbH, Weilheim, Germany; [12,15]). This system was based on four infrared cameras covering the test pen and tracking small reflective marker balls in 3D. Four reflective marker balls (diameter 16 mm, weight 2.6 g) were used as the head target (so-called 6D target) and one ball on each ear as ear targets (so-called 3D targets). The specific configuration of the head target (142 g; fixed by a halter composed of a metal plate on the top of the head of the animal, centred between the ears) allowed identifying the absolute location of the head target in 3D as well as its orientation (pitch, yaw,

and roll angles). The ear targets were fixed singly at the backside of each ear on the earmarks and could be located in absolute 3D space. Based on this data, we calculated the relative position of the ears in relation to the head as expressed by forward/backward and vertical angles at 6 Hz (further details described in [12,15]).

We distinguished between the amount of ear movements (sum of the absolute differences between successive horizontal angles of both ears divided by duration of phase, that is, the duration of pre-stimulus, stimulus, and post-stimulus phases) and four distinct positions of the ears: 1. forward ears (both ears pointed more than 0 degrees forward), 2. backward ears (both ears pointed more than 10 horizontal degrees backward), 3. passive ears (both ears more than 30 vertical degrees below horizontal), and 4. left-asymmetric ears (left ear positioned more than 5 horizontal degrees more forward than right ear per all ear positions with more than 5 degrees difference in their horizontal angle). All positions were calculated as proportions of time within a phase for each single stimulus sequence. Using the tracking of the head target, we also analysed the total locomotor activity (cumulative distance covered by the head divided by length of phase) of the sheep in the test pen. In 26% of the experimental time, no data were logged, due to targets being out of view of the cameras, that is, hidden by body parts of the sheep.

2.8 Habituation of the sheep and experimental procedure

All focal animals had already been habituated to the test pen (in the corner of a building with 1.21-m-high wooden walls on the two remaining sides; Fig. 1) and the measurement equipment in a previous experiment [12] and were re-habituated briefly. The habituation period lasted 6 days, during which each focal sheep was led singly to the test pen once a day. For the first 2 days, the sheep were habituated for 5 minutes to the fixation of the fNIRS sensor (see section 2.5). This fixation included pulling two stockings over the head of the animal for a tight and secure attachment of the sensor. During the following 4 days, the

sheep were habituated additionally to the targets needed for ear tracking. Three times the habituation lasted for 5 minutes and once for 10 minutes. The 10-minute session also included the habituation to the stimulus, that is, the presentation of video sequences of lying or ruminating animals. Because the sheep were familiar with seeing the different types of social interactions in their groups, we refrained from a habituation to all the three different types of stimuli and habituated them only to the new situation of watching videos projected onto a screen and not to the stimuli themselves. All video sequences used in the habituation session were different from those used in the test afterwards. A familiar experimenter was always present with the focal sheep in the experimental pen in order to calm the animal, but the experimenter did not interact with the sheep.

For the experiments, each animal was tested singly in the test pen. Six sheep were tested per day, three from one housing system in the morning and three from the other housing system in the afternoon, alternating mornings and afternoons for the two housing conditions each day. After entering the test pen, the sheep were equipped with the measurement devices (described above and in sections 2.5 and 2.7), followed by 5 minutes of acclimatisation. As during the habituation, a familiar experimenter was always present in the test pen, but did not interact with the sheep.

2.9 Statistical evaluation

All data were modelled by linear mixed-effects models in R versions 2.15.1 and 2.15.3 [35], and we used lmer (package lme4; [36]) for the evaluation of the data. To satisfy statistical assumptions and to choose transformations, graphical analyses of the residuals were conducted. The optimal model was chosen based on the Bayesian information criterion (BIC; [4,12,19]), that is, using model probabilities (*mPr*) for all models investigated. In addition, we report the evidence ratio (E_0) calculated as the ratio of the probability of the chosen model to

the probability of the null model. E_0 therefore indicates how many times the chosen model was more probable than the null model.

In the statistical analysis of the sheep's attentiveness, we calculated the proportion of stimulus sequences in which the sheep were attentive separately for each of the three valences of the stimuli. A logit-transformation was performed on this proportion. The maximum model included the fixed effects housing condition (factor with two levels: predictable and unpredictable), valence of the stimulus, valence squared, and the interactions between housing condition and the valence variables. This coding for valence corresponded to that of an ordered factor and allowed us to drop the quadratic term if changes in response to stimulus valence were not statistically discernible from a linear relationship on the transformed scale. The sheep's identity was included as a random effect.

General aspects of the statistical analyses of the fNIRS data, locomotor activity and ear postures were analogous to the evaluation performed in our previous experiment [12]. In short, total locomotor activity and ear movements of the sheep were log-transformed for the statistical analyses, and all data of the different ear positions were logit-transformed. The maximum models included the fixed effects housing condition (factor with two levels: predictable and unpredictable), valence and its square (ordered factor), phase (factor with three levels: pre-stimulus, stimulus and post-stimulus) and all their potential interactions. The repetition number (stimulation number) nested in stimulus sequence (order of the valences) nested in animal identity served as random effect. The proportion of available data for each phase was used as a weight giving phases with more complete data a higher weight in the evaluation.

The use of lmer allowed an analysis of each single stimulation but did not allow a direct modelling of temporal correlations [18,19]. To correct for this model restriction [12], 3 seconds of the signals were averaged, resulting in four, seven and four values for the pre-stimulus (12 seconds), stimulus (21 seconds) and post-stimulus (12 seconds) phases, respectively. The first second of the post-stimulus interval was combined with the last two

seconds from the stimulus interval for this averaging. The emerging dataset was composed of 12 sheep x 2 housing conditions x 3 types of stimulus valence x 7 repetitions x up to 8 light paths x 15 values throughout each repetition. As a consequence of movement artefacts in single test runs, some stimuli and paths had to be excluded and 96% of the 60,480 potential observations were analysed.

The fNIRS data were evaluated in two steps. The first step was the selection of degrees of freedom for the spline from the numbers 5, 9, and 13 influencing the curvature possible in the model estimates. In the following model selection, with the evaluation beginning from the simplest model being the null model up to the maximum model including all fixed effects (housing condition, stimulus valence, time course and the position on the head) and their interactions, the previously chosen number of degrees of freedom was applied.

3 Results

3.1 Attentiveness

Attentiveness of the sheep decreased monotonously from video sequences showing agonistic interactions to those showing ruminating sheep to co-feeding sheep. Attentiveness was higher in the sheep from the unpredictable, stimulus-poor housing conditions than in those from the predictable, stimulus-rich housing conditions (Fig. 2; model including housing condition and valence: $mPr = 0.23$, $E_0 = 5.07$). Additionally, models with housing condition and valence squared ($mPr = 0.22$, $E_0 = 4.83$), and housing condition only ($mPr = 0.20$, $E_0 = 4.39$) reached similar model probabilities supporting the clear difference between sheep from the different housing conditions and the monotonous effect of the three stimuli.

3.2 Frontal brain activity

The smallest number of degrees of freedom (df = 5) for modelling the natural spline of the time course was sufficient for [O_2Hb] and [HHb] (*mPr* = 1.00).

No effect of the predictor variables was apparent for [O_2Hb]; accordingly, the null model was the most probable (Fig. 3a; *mPr* = 0.99).

There was strong evidence that [HHb] was influenced by the interaction of housing condition, stimulus valence, and time course (*mPr* = 1.00, E_0 = 6758). [HHb] showed a strong peak when sheep from the predictable, stimulus-rich housing conditions watched the videos of other sheep showing agonistic interactions (Fig. 3b).

3.3 Behavioural measurements: locomotor activity and ear postures

Strong evidence was found that the locomotor activity of sheep from the unpredictable, stimulus-poor housing conditions was about three times as high as that of sheep from the predictable, stimulus-rich housing conditions during all three types of stimulus valence (strongest model including the main effect housing condition: *mPr* = 0.88, E_0 = 883). A higher activity during the stimulus and post-stimulus phases compared to the pre-stimulus phases was only supported more weakly (model additionally including phase: *mPr* = 0.06, E_0 = 62; Fig. 4a). The strongest model of the ear movement data included the intercept only (*mPr* = 0.82) and showed only weak evidence that more ear movements were shown by the sheep from the unpredictable, stimulus-poor housing conditions compared with the sheep from the predictable, stimulus-rich housing conditions (*mPr* = 0.11, E_0 = 0.14; Fig. 4b).

The proportion of forward ears was generally low and increased from the pre-stimulus to the stimulus phase (model including phase: *mPr* = 0.65, E_0 > 1300). Animals from the predictable, stimulus-rich housing conditions showed more forward ears than those from the unpredictable, stimulus-poor housing conditions, with an additional slight increase from the

stimulus to the post-stimulus phase (model including the main effects phase and housing condition and their interaction: *mPr* = 0.21, E_0 > 420; Fig. 4c). The reverse pattern was detected in the proportion of backward ears, which decreased from the pre-stimulus to the stimulus to the post-stimulus phase (strongest model including phase: *mPr* = 0.41, E_0 = 4.87) and was higher in sheep from the unpredictable, stimulus-poor housing conditions than in sheep from the predictable, stimulus-rich housing conditions (model including additional effect housing condition: *mPr* = 0.35, E_0 = 4.21; Fig. 4d). There was only weak evidence that the proportion of passive ears increased across the three phases (model including phase: *mPr* = 0.31, E_0 = 0.53; model including intercept only: *mPr* = 0.59; Fig. 4e). There was no evidence that the proportion of left forward ears was influenced by the type of stimulus, the housing conditions or the phase (model including intercept only: *mPr* = 0.88; Fig. 4f).

4 Discussion

In the present study, no consistent reactions discerning the three stimuli could be observed in the sheep's locomotor activity, ear movement, ear postures, or frontal brain reaction. Sheep responded to the three presented stimuli in that their attentiveness decreased from video sequences showing agonistic interactions to those showing ruminating sheep to the affiliative interactions. The differences in attentiveness were consistent with the presumed valence of each stimulus and supported our hypothesis that sheep would decrease their attentiveness from the negative to the positive social stimulus. However, an alternative explanation is possible. During the agonistic interactions, the rate of movements, that is, how often the projected video images changed per time, was higher than during the other two stimuli. High motion rates have previously been found to increase attentiveness [22,37] and could explain at least part of the pattern. Given that the relationship of attentiveness with the three stimuli was monotonous and followed a concave downward curve from agonistic interactions to ruminating behaviour to affiliative interactions, the rate of movement cannot be the sole explanation, and we conclude that a combination of valence and movement rate

induced the observed pattern of attentiveness. Compared with previous studies [12,14], the complete lack of differences between the stimuli's effects on locomotor activity, ear movement and ear posture seems surprising. It could be explained by the fact that these behavioural expressions are intra-specific signals. Hence, regardless of the valence, the animals may always pay attention and react to certain stimuli of social interactions gaining information resulting in individual benefits [38]. Alternatively, one can speculate, that it is potentially costly or disadvantageous to signal an emotional reaction in a social context.

The results of the current study indicated many general effects of housing conditions in that sheep from the predictable, stimulus-rich housing conditions were less attentive, less active, moved their ears less, and had their ears forward more often and backward less often than the sheep from the unpredictable, stimulus-poor housing conditions. These differences would be consistent with the notion that a relatively negative mood was induced by the unpredictable, stimulus-poor conditions [23,24] and a relatively more positive mood was induced by the predictable, stimulus-rich conditions [23,25,26]. However, a cognitive judgement bias test, performed 9 weeks before the present experiment [12], indicated only weak differences between the two groups of sheep in their decision behaviour towards ambiguous stimuli. As suggested there, the stimulus richness of the testing procedure could have elicited specifically the differences in locomotor activity if the sheep from the unpredictable, stimulus-poor conditions were either more challenged by these stimuli [12] or more motivated to explore a stimulus-rich environment [39,40] than the sheep from the predictable, stimulus-rich conditions, which were well habituated to a variety of stimuli. In respect to attentiveness, the larger dimensions of the predictable, stimulus-rich housing system may have allowed the animals to evade more easily when other animals were fighting [41]. In contrast, the animals living under barren and stimulus-poor housing conditions might have needed to be more vigilant to avoid an involvement in a fight given the limited space allowance [42].

The differences in forward and backward ear postures would be consistent with the interpretation that the sheep from the unpredictable, stimulus-poor conditions were in a more negative affective state and expressed more behaviour indicating negative emotions compared with the sheep from the predictable, stimulus-rich conditions, as several studies associated backward ears with a negative situation [11,13,43]. This finding contrasts our previous results [12,14], where backward ears were found during positive rather than negative situations. A possible explanation for this difference between studies may be that ear postures depend not only on the valence of the stimuli but also on the way the stimuli were presented. While the stimuli in the present study and the studies by Boissy et al. [13], Guldimann et al. [20], and Veissier et al. [44] were presented visually, the stimuli in the studies by Reefmann et al. [14] and Vögeli et al. [12] were physical and perceived by the touch receptors.

In contrast to the recorded behavioural data, the fNIRS data of the present study showed that housing conditions modulated the emotional responses of the sheep to the social stimuli. The sheep from the predictable, stimulus-rich housing conditions in our experiment specifically exhibited an increase in [HHb] while watching the videos of two animals engaged in agonistic interactions. Neuronal activation leads to a decrease in [HHb] and an increase in [O_2Hb] [45]. Hence it can be assumed that brain activity was reduced at the site of measurement [46]. This contrasts previous studies that found frontal brain activation in response to more negative stimuli [12,19]. These stimuli were non-visual suggesting that the mode of presentation could influence brain reactions. A study in humans indeed also exhibited a deactivation of the dorsal prefrontal cortex when subjects were playing video games, which the researchers associated with a neuronal inhibition caused by an attention demand required for visual stimuli [47]. A negative correlation between visual attention load and brain activity was also found by Mazoyer [48], who showed by functional magnetic resonance imaging in an experiment with humans that the activity in the medial superior frontal cortex specifically decreased while the attention load towards visual stimuli increased. The deactivation found in our experiment could therefore be caused by such an attention demand

(i.e. ‘vascular steal’; [49]) resulting from observing the video sequences on screen. Nevertheless, according to this interpretation, a deactivation should have been detected in sheep from both housing systems and not predominantly in sheep from the predictable, stimulus-rich housing conditions. All in all, when we try to reconcile this pattern with previous hypotheses on the interaction of mood and emotional reactions, there is no support for either of the hypotheses. Emotional reactions to videos showing social interactions did not seem to be generally tainted in the sheep from the unpredictable, stimulus-poor housing conditions, nor was the reaction towards a negative stimulus mitigated in the sheep from the predictable, stimulus-rich conditions.

A cortical brain reaction was found for the presumed negative stimulus in the sheep from the predictable, stimulus-rich housing conditions whereas a general effect of the housing condition independent of stimulus valence was found in the locomotor activity, ear movements, and ear positions. This does not suggest that there was a close relationship between the brain and the behavioural reactions in the current study.

In principle, the sheep may have perceived the valence of stimuli in a sequence opposite to our initial hypothesis: the observation of two animals fighting could lead to a positive emotion similar to the relief of not being involved in the fight, and it could be frustrating to observe other sheep feeding without having access to feed. Although this allocation of valence values to the stimuli might explain the results of the fNIRS measurements in terms of a deactivation during a presumed positive stimulus [18,21], it is more difficult to reconcile with our observations of attentiveness. The reverse scoring of the valence would indicate a decreased attentiveness towards the negative stimulus, which does not seem plausible. Therefore, the original assumption on the valence of the three types of social interactions seems to be more likely and is consistent with previous studies on chimpanzees [29] and humans [32]. In future studies, it could be useful to try to have an independent assessment of the valence of the stimuli, for example, by conducting preference tests in respect to the different stimuli used.

The experimental sheep were familiar with the animals visible in the video sequences, that is, they were from the same housing group. This was relevant because sheep can identify familiar sheep [50] and the interactions in the video scenes were therefore expected to be more meaningful for the observing animals [51]. However, this familiarity could also have modified the emotional reactions depending on the detailed relationship between the watching animal and those visible on screen, in addition to the effect of the general type of the visible social interactions. To minimise this potential bias, the seven 20 sec clips per valence each consisted of three to four shorter clips involving a variety of familiar sheep.

The animals in the present experiment had been living under their assigned housing conditions for several months. During that time, they may have gotten used to the housing conditions, even to the unpredictable, stimulus-poor conditions, virtually having reset their baseline mood. Therefore, a change in conditions may have had a larger impact on their mood than the constant long-term chronic conditions. Furthermore, the different types of social interactions were all presented by video sequences, contrasting with earlier experiments that, for example, directly applied physical stimuli [4,12]. Possibly, the way of presenting the stimuli may influence the animal's reaction in addition to their valence. Finally, the range of the elicited emotional reactions in this experiment may not have been sufficient to make a strong inference on the emotional reactions of the sheep and to depict mood-emotion interactions.

5 Conclusions

Sheep discriminated between video sequences presenting different types of social interactions of conspecifics. Agonistic interactions had the greatest importance for the animals in that they elicited the highest level of attentiveness. Predictability and stimulus-richness of the housing conditions had additional consequences on the behaviour of the sheep during testing, although it remains unclear whether these differences were mediated

by mood or whether the previous experience with stimuli in their home pen more directly changed the reactions when the sheep were confronted with the test stimuli. It seems that frontal cortical activity does not only depend on the valence but also on the way a stimulus is presented, for example visual presentation versus direct physical stimuli.

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7 References

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Figure captions

Figure 1: Test pen with an experimental sheep equipped with the measurement instruments. The white rectangle indicates the video screen.

Figure 2: Attentiveness of the sheep during the stimulus video sequences as a function of the sheep's housing conditions (unpredictable and predictable) and the stimulus valence. Box plots indicate the data range, the median, and the lower and upper quartiles. Thick black lines: model estimates; thin black lines: 95% confidence intervals.

Figure 3: Average changes in [O_2Hb] (a) and [HHb] (b) throughout the application of three different stimulus video sequences as a function of the sheep's housing conditions (unpredictable and predictable). Thick black lines: model with highest model probability (interaction of housing condition, stimulus valence and time); thin black lines: 95% confidence intervals.

Figure 4: General activity (distance covered, m/s; a), ear movements (degrees/s; b), proportion of forward (c) and backward (d) ear postures, proportion of time that ears were passive (e), and proportion of time the left ear was further to the front than the right ear (f) as a function of the sheep's housing conditions (unpredictable and predictable), the stimulus type, and the phase of the stimulus (Pr = pre-stimulus, S = stimulus, Po = post-stimulus). Box plots indicate the data range, the median, and the lower and upper quartiles. Thick black lines: model estimates; thin black lines: 95% confidence intervals. Y-axes are cropped in a and b to enhance visibility of the pattern reflected by the statistical estimates.

Figure 1:

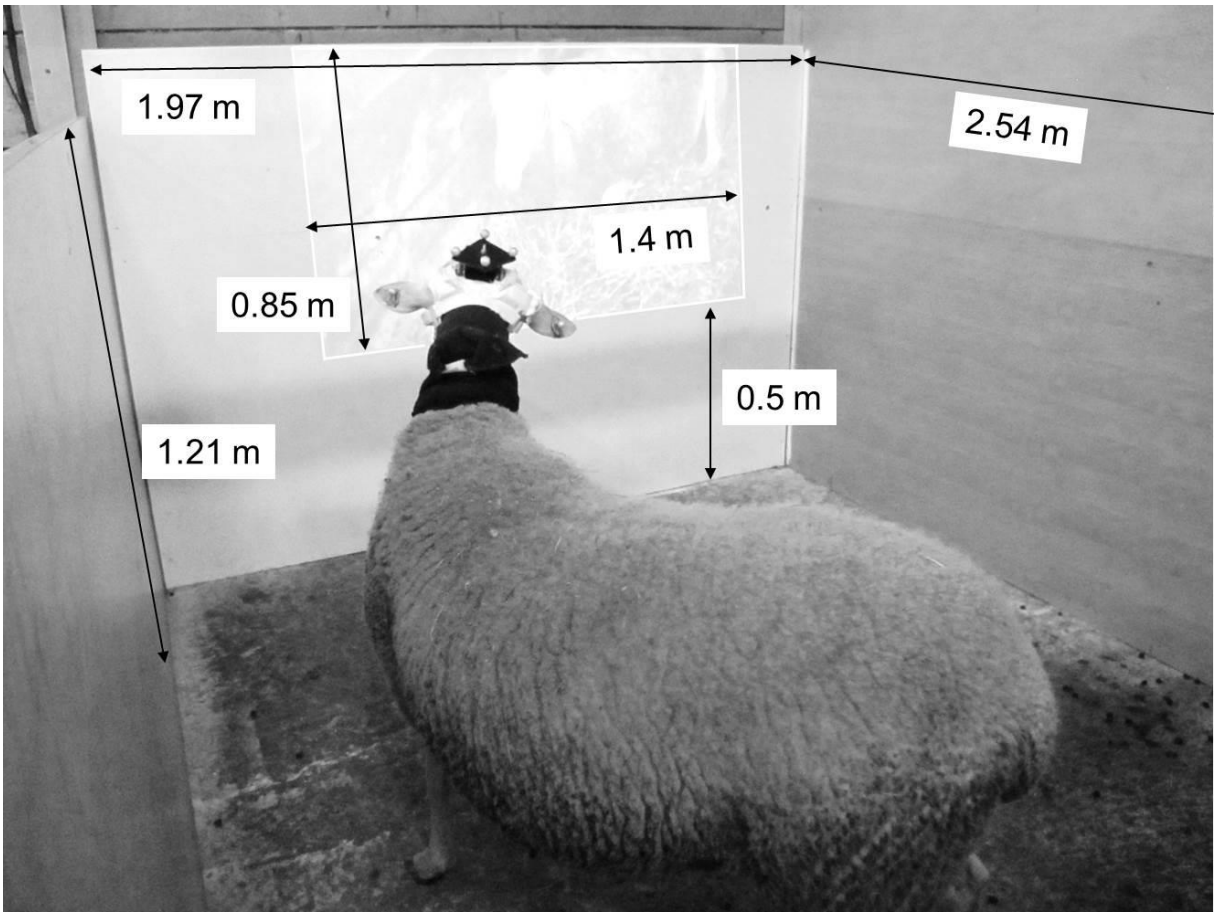


Figure 2:

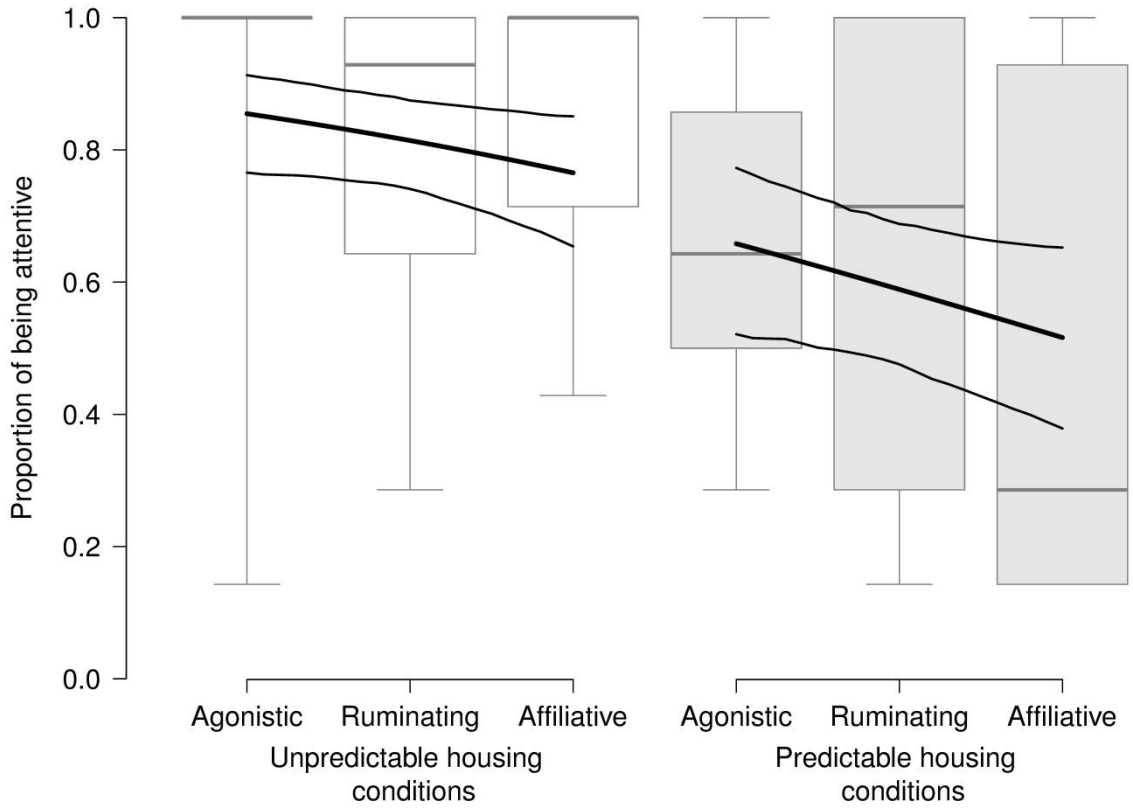

1.0
0.8
0.6
0.4
0.2
0.0
Proportion of being attentive
Agonistic
Ruminating
Affiliative
Unpredictable housing conditions
Agonistic
Ruminating
Affiliative
Predictable housing conditions

Figure 3:

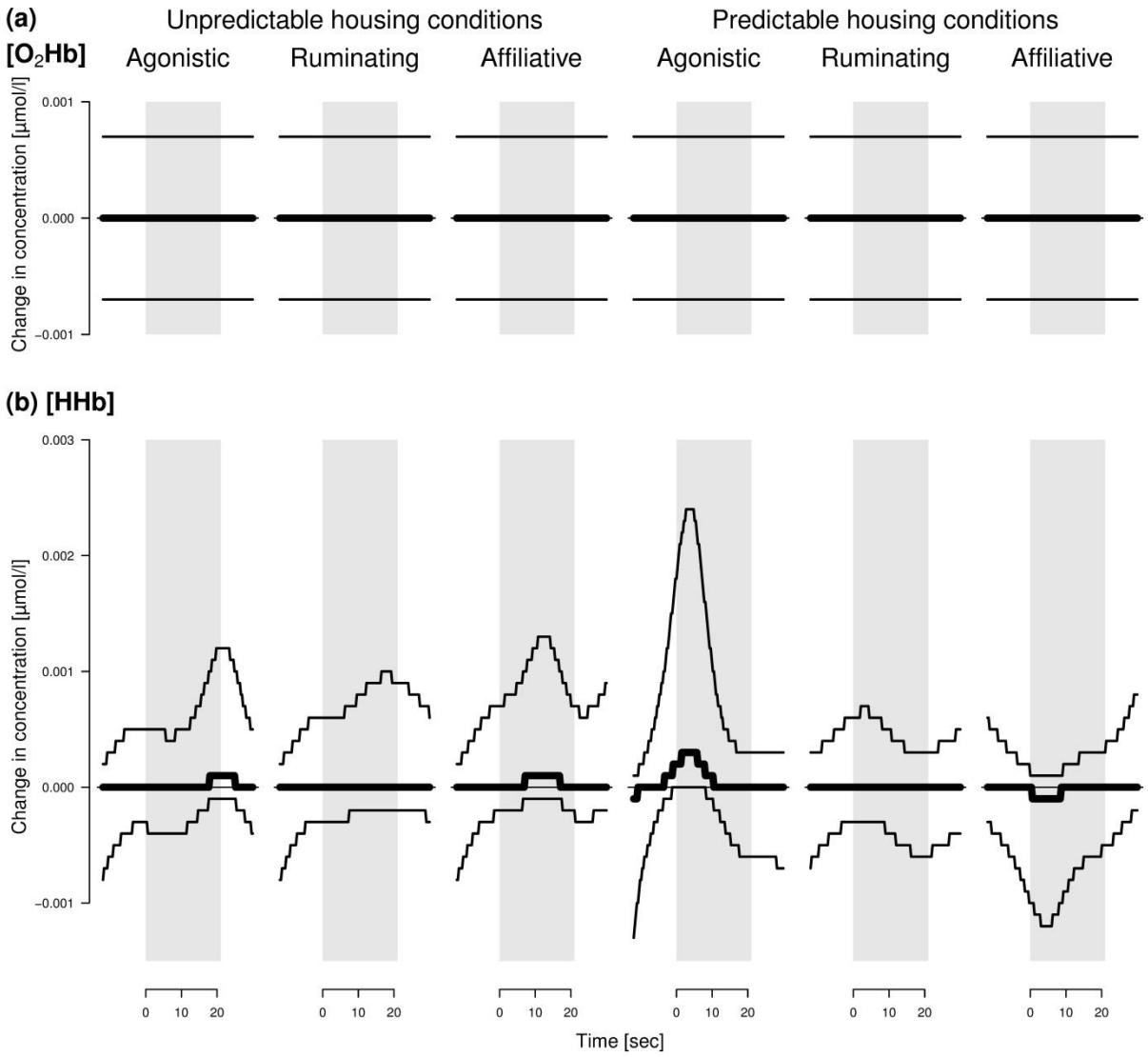
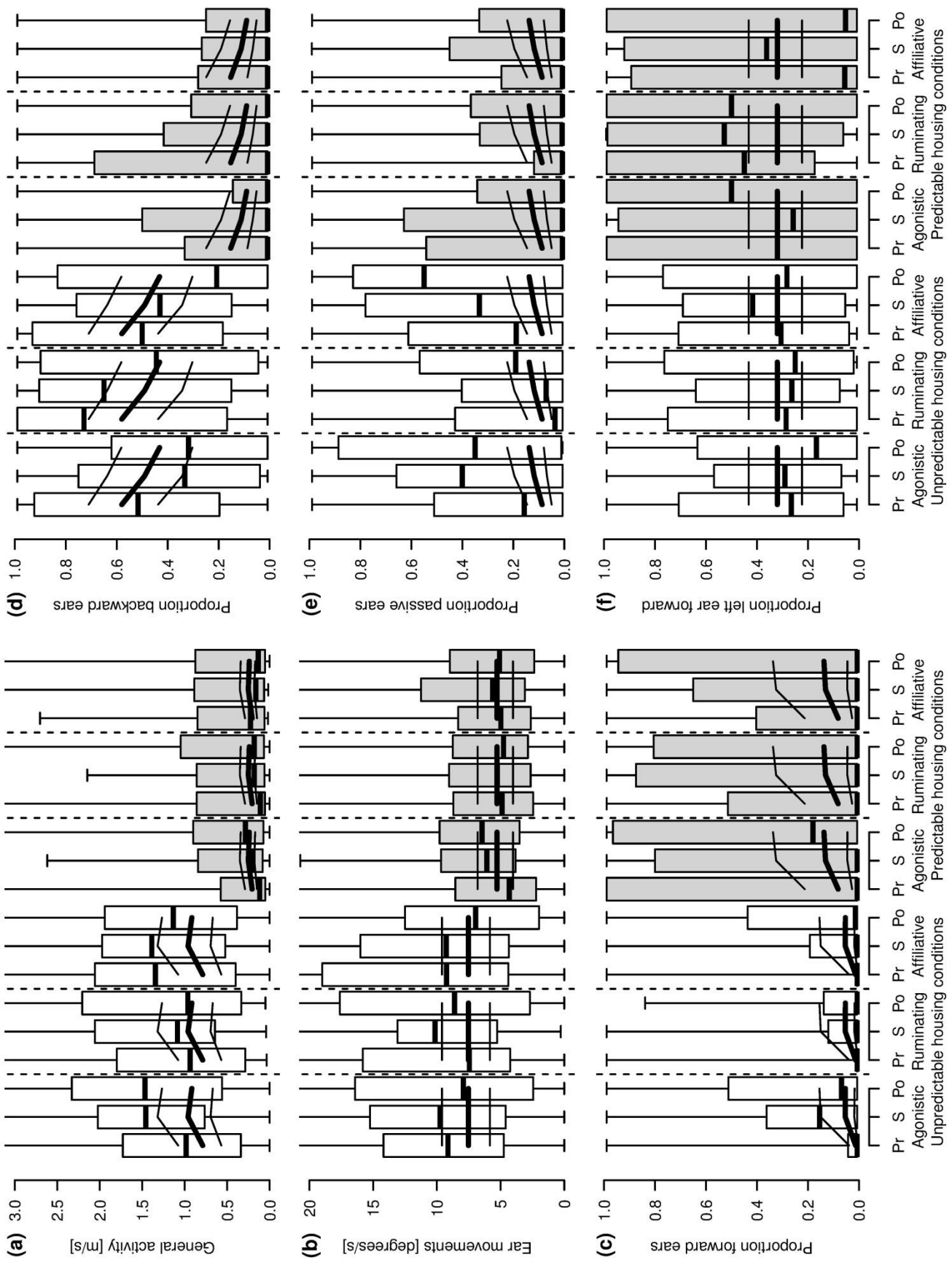

(a)
[O2Hb]
Unpredictable housing conditions
Predictable housing conditions
Agonistic
Ruminating
Affiliative
Agonistic
Ruminating
Affiliative
Change in concentration [µmol/l]
0.001
0.000
−0.001
(b) [HHb]
0.003
0.002
0.001
0.000
−0.001
0
10
20
Time [sec]

Figure 4:


(a)
General activity [m/s]
(b)
Ear movements [degrees/s]
(c)
Proportion forward ears
(d)
Proportion backward ears
(e)
Proportion passive ears
(f)
Proportion left ear forward
Pr S Po Pr S Po Pr S Po Pr S Po
Agonistic Ruminating Affiliative Agonistic Ruminating Affiliative
Unpredictable housing conditions
Predictable housing conditions